

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows.

Please cancel claims 101, 103, 106, 107, 117 to 121 and 136 to 138 without prejudice or disclaimer.

This listing of claims will replace all prior versions, and listing, of claims in the application.

Claims 1 to 41 (canceled)

Claim 42 (currently amended): A method of generating a nucleic acid encoding an endoglucanase comprising:

obtaining a nucleic acid encoding an endoglucanase comprising a sequence having at least about 95% ~~[[70%]]~~ sequence identity to ~~a sequence as set forth in~~ SEQ ID NO:1, or sequences complementary thereto; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence.

Claim 43 (currently amended): The method of claim 42, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, Gene Site Saturation Mutagenesis (GSSM) ~~MutagenesisTM~~ (~~GSSMTM~~) and any combination thereof.

Claim 44 (original): The method of claim 42, wherein the modifications are introduced by error-prone PCR.

Claim 45 (original): The method of claim 42, wherein the modifications are introduced by shuffling.

Claim 46 (original): The method of claim 42, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

Claim 47 (original): The method of claim 42, wherein the modifications are introduced by assembly PCR.

Claim 48 (original): The method of claim 42, wherein the modifications are introduced by sexual PCR mutagenesis.

Claim 49 (original): The method of claim 42, wherein the modifications are introduced by *in vivo* mutagenesis.

Claim 50 (original): The method of claim 42, wherein the modifications are introduced by cassette mutagenesis.

Claim 51 (original): The method of claim 42, wherein the modifications are introduced by recursive ensemble mutagenesis.

Claim 52 (original): The method of claim 42, wherein the modifications are introduced by exponential ensemble mutagenesis.

Claim 53 (original): The method of claim 42, wherein the modifications are introduced by site-specific mutagenesis.

Claim 54 (original): The method of claim 42, wherein the modifications are introduced by gene reassembly.

Claim 55 (currently amended): The method of claim 42, wherein the modifications are introduced by Gene Site Saturation Mutagenesis (GSSM) ~~MutagenesisTM (GSSMTM)~~.

Claims 56 to 87 (canceled)

Claim 88 (currently amended): A method for modifying an endoglucanase substrate ~~small molecules~~, comprising

providing a polypeptide encoded by a polynucleotide comprising a sequence having at least about 95% ~~[[70%]]~~ identity to SEQ ID NO:1 and encoding a polypeptide having an endoglucanase enzymatic activity,

providing an endoglucanase substrate ~~a small molecule~~; and

mixing the polypeptide with the endoglucanase substrate ~~small molecule~~ to produce a modified endoglucanase substrate ~~small molecule~~.

Claim 89 (currently amended): The method of claim 88 wherein the modified endoglucanase substrate ~~small molecule~~ is tested to determine if it exhibits a desired activity.

Claim 90 (currently amended): The method of claim 89 wherein a library of modified endoglucanase substrates ~~small molecules~~ are made from a single starting endoglucanase substrate ~~small molecule~~ compound in a plurality of biocatalytic reactions, and the specific biocatalytic reaction which produces the modified endoglucanase substrate ~~small molecule~~ of desired activity is identified by systematically eliminating each of the biocatalytic reactions used to produce the modified endoglucanase substrate ~~small molecule~~ library, and then testing the endoglucanase substrates ~~small molecules~~ produced in the portion of the library for the presence or absence of the modified endoglucanase substrate ~~small molecule~~ with the desired activity.

Claim 91 (currently amended): The method of claim 90 wherein the specific biocatalytic reactions which produce the modified endoglucanase substrate ~~small molecule~~ of desired activity is optionally repeated.

Claim 92 (currently amended): The method of claim 91 wherein the biocatalytic reactions are conducted with a group of biocatalysts that react with distinct structural moieties found within the structure of the endoglucanase substrate ~~a small molecule~~, each biocatalyst is specific for one structural moiety or a group of related structural moieties; and each biocatalyst reacts with many different endoglucanase substrates ~~small molecules~~ which contain the distinct structural moiety.

Claim 93 (currently amended): A method of generating a nucleic acid encoding an endoglucanase comprising:
obtaining a nucleic acid encoding an endoglucanase, wherein the nucleic acid comprises at least 50 ~~[[75]]~~ consecutive residues of a sequence having at least about 95% ~~[[70%]]~~ sequence identity to a sequence as set forth in SEQ ID NO:1 or sequences complementary thereto; and
modifying one or more nucleotides in the sequence to another nucleotide, deleting one or more nucleotides in the sequence or adding one or more nucleotides to the sequence.

Claim 94 (previously presented): A method of generating a nucleic acid encoding an endoglucanase comprising:
obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:1 or sequences complementary thereto; and
modifying one or more nucleotides in the sequence to another nucleotide, deleting one or more nucleotides in the sequence or adding one or more nucleotides to the sequence.

Claim 95 (currently amended): A method for modifying an endoglucanase substrate ~~a small molecule~~ comprising:

providing a polypeptide having an endoglucanase enzymatic activity, wherein the polypeptide is encoded by a nucleic acid comprising at least 75 consecutive residues of a sequence having at least about 95% [[70%]] sequence identity to a sequence as set forth in SEQ ID NO:1;
providing an endoglucanase substrate ~~a small molecule~~; and
mixing the polypeptide with the endoglucanase substrate ~~small molecule~~ to produce a modified endoglucanase substrate ~~small molecule~~.

Claim 96 (currently amended): A method for modifying an endoglucanase substrate ~~a small molecule~~ comprising:

providing a polypeptide having an endoglucanase enzymatic activity, wherein the polypeptide is encoded by a nucleic acid comprising a sequence as set forth in SEQ ID NO:1;
providing an endoglucanase substrate ~~a small molecule~~; and
mixing the polypeptide with the endoglucanase substrate ~~small molecule~~ to produce a modified endoglucanase substrate ~~small molecule~~.

Claims 97 to 109 (canceled)

Claim 110 (currently amended): The method of claim 126, wherein the endoglucanase enzymatic activity comprises a carboxymethyl cellulase activity or hydrolysis of the beta 1,4 glycosidic bonds.

Claim 111 (currently amended): A method of generating and identifying a nucleic acid encoding a polypeptide having endoglucanase enzymatic activity comprising:

obtaining a nucleic acid encoding an endoglucanase comprising a sequence having at least about 95% [[70%]] sequence identity to at least 30 consecutive residues of a sequence as set forth in SEQ ID NO: 1 or sequences complementary thereto;

modifying one or more nucleotides in the sequence to another nucleotide, deleting one or more nucleotides in the sequence, or adding one or more nucleotides to the sequence; and

identifying a modified nucleic acid encoding a polypeptide having endoglucanase enzymatic activity.

Claim 112 (currently amended): A method for modifying an endoglucanase substrate a ~~small-molecule~~ such that the endoglucanase substrate ~~small-molecule~~ will have a desired activity comprising:

providing a polypeptide having endoglucanase enzymatic activity, wherein the polypeptide is encoded by a nucleic acid having at least about 95% ~~[[70%]]~~ sequence identity to at least 30 consecutive residues of a sequence as set forth in SEQ ID NO:1;

providing a endoglucanase substrate ~~small-molecule~~;

mixing the polypeptide with the endoglucanase substrate ~~small-molecule~~ to produce a modified endoglucanase substrate ~~small-molecule~~; and,

testing the modified endoglucanase substrate ~~small-molecule~~ for the desired activity.

Claim 113 to 114 (canceled)

Claim 115 (currently amended): The method of claim 88, wherein mixing the polypeptide with the endoglucanase substrate ~~small-molecule~~ produces a library of modified endoglucanase substrates ~~small-molecules~~.

Claim 116 (currently amended): The method of claim 115, wherein the library of modified endoglucanase substrates ~~small-molecules~~ is tested to determine if a modified endoglucanase substrate ~~small-molecule~~ exhibiting a desired activity is present within the library, wherein optionally the desired activity comprises hydrolysis of the beta 1,4 glycosidic bonds.

Claims 117 to 121 (canceled)

Claim 122 (previously presented): The method of claim 42, wherein the sequence identity is 97%.

Claim 123 (currently amended): A method of generating a nucleic acid encoding an endoglucanase comprising:

obtaining a nucleic acid encoding an endoglucanase, wherein the nucleic acid comprises a sequence that (a) hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, and the stringent hybridization conditions comprise hybridizing the nucleic acid to an immobilized denatured SEQ ID NO:1 at 45°C in a solution comprising 0.9M NaCl, 50 mM NaH₂PO₄, pH 7.0, 5.0 mM Na₂EDTA, 0.5% SDS, 10x Denhardt's and 0.5 mg/mL polyriboadenylic acid for 12 to 16 hours, and a wash step comprising washing for 30 minutes at room temperature in a solution comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS, followed by a 30 minute wash in fresh solution, or (b) sequences complementary to (a); and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence.

Claim 124 (currently amended): A method for modifying endoglucanase substrates ~~small molecules~~, comprising

providing a polypeptide having endoglucanase enzymatic activity encoded by a nucleic acid comprising a sequence that (a) hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, and the stringent hybridization conditions comprise hybridizing the nucleic acid to an immobilized denatured SEQ ID NO:1 at 45°C in a solution comprising 0.9M NaCl, 50 mM NaH₂PO₄, pH 7.0, 5.0 mM Na₂EDTA, 0.5% SDS, 10x Denhardt's and 0.5 mg/mL polyriboadenylic acid for 12 to 16 hours, and a wash step comprising washing for 30 minutes at room temperature in a solution comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS, followed by a 30 minute wash in fresh solution,

providing an endoglucanase substrate ~~a small molecule~~; and

mixing the polypeptide with the endoglucanase substrates ~~small molecule~~ to produce a modified endoglucanase substrate ~~small molecule~~.

Claim 125 (currently amended): The method of claim 123 or claim 124, wherein the endoglucanase activity comprises a carboxymethyl cellulase activity or is capable of hydrolyzing beta 1,4 glycosidic bonds.

Claim 126 (currently amended): A method for modifying an endoglucanase substrate a ~~small molecule~~ comprising:

providing a polypeptide having an endoglucanase enzymatic activity, wherein the polypeptide is encoded by a nucleic acid comprising at least 30 consecutive residues of a sequence having at least about 95% sequence identity to a sequence as set forth in SEQ ID NO:1;

providing an endoglucanase substrate ~~a small molecule~~; and

mixing the polypeptide with the endoglucanase substrate ~~small molecule~~ to produce a modified endoglucanase substrate ~~small molecule~~.

Claim 127 (currently amended): The method of claim 126, wherein the polypeptide is encoded by a nucleic acid comprising at least 50 ~~[[40]]~~ consecutive residues of a sequence having at least about ~~85%, 90%, 95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO:1.

Claim 128 (currently amended): The method of claim 111, wherein the nucleic acid encoding an endoglucanase comprises at least 50 ~~[[30]]~~ consecutive residues of a sequence having at least about ~~95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO: 1.

Claim 129 (currently amended): The method of claim 111, wherein the nucleic acid encoding an endoglucanase comprises at least 40 consecutive residues of a sequence having at least about ~~85%, 90%, 95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO: 1.

Claim 130 (currently amended): The method of claim 111, wherein the nucleic acid encoding an endoglucanase comprises at least 75 consecutive residues of a sequence having at least about ~~70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO:1.

Claim 131 (currently amended): The method of claim 112, wherein the polypeptide having endoglucanase enzymatic activity is encoded by a nucleic acid comprising at least 50 ~~[[30]]~~ consecutive residues of a sequence having at least about ~~95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO: 1.

Claim 132 (currently amended): The method of claim 112, wherein the polypeptide having endoglucanase enzymatic activity is encoded by a nucleic acid comprising at least 40 consecutive residues of a sequence having at least about ~~85%, 90%, 95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO: 1.

Claim 133 (currently amended): The method of claim 112, wherein the polypeptide having endoglucanase enzymatic activity is encoded by a nucleic acid comprising at least 75 consecutive residues of a sequence having at least about ~~70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO: 1.

Claim 134 (currently amended): The method of claim 93, wherein the nucleic acid comprises ~~at least 75 consecutive residues of~~ a sequence having at least about 97% ~~[[75%]]~~ sequence identity to a sequence as set forth in SEQ ID NO:1 or sequences complementary thereto.

Claim 135 (currently amended): The method of claim 93 ~~[[134]]~~, wherein the nucleic acid comprises at least 75 consecutive residues of a sequence having at least about ~~80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%~~ sequence identity to a sequence as set forth in SEQ ID NO:1 or sequences complementary thereto.

Claim 136 to 138 (canceled)